Attachment 7. **Field Research Plan**

1. Construction and design of the pilot CWTS.

Location. The pilot CWTS will be located in an approximately 10-15 acre exposed playa near the mouth of Alamo River discharging into the Salton Sea to the south (33°11'56.73"N, 115°35'57.18"W). Its borders are the Alamo River to the north, Red Hill Bay Road to the south, Garst Road to the east and Red Hill to the west (see *Attachment 8, Figure 1*). The site is an exposed playa whose slope is less than 0.1%. The average elevation on the property is approximately 71 m below mean sea level.

Site characteristics. The site is an exposed playa and currently not in use for any specific purpose. The site has no existing utilities. The major utilities in adjacent roads consist of agricultural drains and electricity that can be used to power the pumps and other facilities after wetland construction. According to data of the Calipatria CIMIS station (1990-2009), temperatures in the southern Salton Sea area typically average 13°C in the winter and 32 °C in the summer. Rainfall in this area is usually very low, at 7 cm/year, while average evapotranspiration rate is high, at 1.8 m/year. Rain occurs predominantly from November through March. Because evapotranspiration rates are substantially higher than rainfall, residence time of the water in the wetland should be kept to a minimum to maximize water flows to the SCH especially during summer.

Construction and layout. The pilot CWTS will be constructed by the Imperial Irrigation District (IID), which will be responsible for obtaining the necessary permits, site survey and preparation, final design, wetland construction, facility installment, including electricity, pumps, weirs, and pipes. After construction, IID will share the responsibility with UC Berkeley to operate and maintain the CWTS for the next three years. The layout of the CWTS, which will consist of a sedimentation pond (30-m x 50-m), eight long-length treatment cells (each 6-m x 120-m) and eight short-length cells (6-m x 60-m), is illustrated in *Attachment 8, Figure 2*. The cells will be constructed according to the general design criteria (*Attachment 8, Table 1*). All cell inlets will consist of 5-cm PVC irrigation pipe with a PVC butterfly control valve and 5-cm impeller flow meter installed inside the inlet pipe to regulate and monitor inlet flow. Outlets will consist of 25-cm galvanized metal pipe with measured v-notch weir.

Initial CWTS set-up. After construction of the CWTS, each cell will be filled with river water to a level of 0.5 meter. The bottom of each cell will be disturbed to re-suspend the fine particles and the cell refilled with water to the top of the bank. The cells will be left alone for at least a day to allow gravitational settling that will sort particles by grain size (see *Attachment 8, Figure 3)*. This act will be repeated at least two more times to create a clay liner at the surface of the bottom to prevent seepage. Once the clay liner is properly formed (i.e., when the water level remains constant indicating that there is no more seepage), the water level will be adjusted to a level of 10 cm to keep the clay liner wet and intact. The cattail treatment cells will be filled with a 20 cm layer of cattail shoots harvested from drains nearby. In constructed wetlands, the fallen cattail litter layer of the upper sediment substrate is required to provide habitat and nutrients for the microbes which carry out the microbial transformations essential for pollutant removal. Because it normally takes some time for a wetland to mature and form a substantial litter layer, we intend to overcome this problem by using locally available cattail shoots.

Planting plan. The cattail treatment cells will be flooded and transplants of cattails collected from nearby wetlands will be planted by hand at a plant spacing of 1 m square. Cattail (*Typha latifolia*) was selected as the target wetland plant for several reasons. It has a high rate of biomass production, which is important for providing fallen litter and other organic material essential for microbial Se volatilization and pollutant immobilization. It is also highly competitive under field conditions, and tends to dominate over other wetland plant species in the Salton Sea area. After planting, river water will be pumped (at a low speed of 1,000-3,000 gallons/day) through each of the cattail-planted cells for the next four months. For the algal species experiment, the eight short-length algal treatment cells will be planted with different algal species including *Chlorella* sp, *Scenedesmus* sp and two local algal species. The results obtained from this experiment will be used to determine the algal species and algal population density to be used in the four main algal-cattail treatments.

2. Initial experimental plan.

Experiment 1: Determination of minimum residence time. After successful establishment of the cattail-planted cells (i.e., at about four months from transplantation), we will use the four long-length algal-cattail treatment cells to determine the minimum residence time needed to reduce Se and fertilizer nutrients to the required low levels. Residence times of 3, 6, 9 and 12 days will be tested by manipulating water depth (5-60 cm for cattail cell and 20-90 cm for algal cells) and hydraulic loading rate. Selenium removal efficiency and nutrient removal efficiency will be monitored as described below.

Experiment 2. Quantitative evaluation of the algal treatment cell component. To test the concept that the inclusion of an algal treatment cell will result in high rates of Se volatilization and therefore less accumulation of Se in the CWTS ecosystem, the selected algal species will be added to the four long-length algal treatment cells at four different population densities (including zero algae added for one of the cells). In addition to determining the efficiencies of Se and nutrient removal, the algal density of each algal cell will be monitored monthly and maintained at constant levels (as far as possible) throughout the experimental period. Quantitative measures of Se volatilization occurring in each of the cattail treatment cells will be estimated every three months by mass balance.

Experiment 3. Algal species comparison. After the clay liner is in place, we will grow different algal species in the eight short-length algal treatment cells for one month. Initially, we will compare four different species of algae (*Chlorella* sp, *Scenedesmus* sp and two local algal species) in terms of their efficiency in removing Se from river water through Se volatilization and through Se accumulation in their biomass, as well as the removal of fertilizer nutrients. Each species will be grown at different population densities to determine the effect of population density on Se removal. The raceway pond will be installed with a paddle wheel to ensure adequate circulation (the water depth and the paddling speed will be adjusted to create the most appropriate mixing and aeration conditions for algal growth and pollutant removal). Algal growth will be monitored by measuring chlorophyll concentration. Removal efficiencies of Se and nutrients will be monitored by collecting water samples for total Se, N, P and S, at the inlets and outlets of each algal cell each month. To verify the mass balance estimates of Se volatilization in the algal cells, volatilization rates will be measured under laboratory conditions at UC Berkeley.

Experiment 4. Changes with wetland maturity. As the cattail wetland matures over the three-year period, two different types of change are likely to take place – changes in pollutant removal efficiency and changes in the accumulation of pollutants within the wetland ecosystem. Firstly, pollutant removal efficiency should increase as the fallen cattail litter and other organic matter in the sediments accumulate. This in turn will lead to a substantial increase in microbial habitat and activity, which should result in increased rates of Se removal (through volatilization and immobilization). Secondly, even though the introduction of the algal treatment cell should lead to considerable losses of Se to the atmosphere through volatilization, there will nevertheless be some buildup of Se in the sediments. Depending on the chemical species of Se building up in the sediments, there is a potential risk of Se ecotoxicity.

In Experiment 4 we will determine the changes over time in the removal efficiency of the pollutants, Se, N, P and S between the inlets and outlets of the CWTS on a monthly basis over the three-year experimental period. In order to assess the changes taking place in the wetland sediments over time we will measure soil organic matter (SOM) and the changes in the thickness of the microbial biofilm (consortium) on the surfaces of the fallen litter layer. This will be carried out monthly by 2-cm dia profiling of the sediments to a depth of 25 cm. These results will enable us to establish the correlative dependence of pollutant removal efficiency on SOM and microbial populations within the sediments. Such information is an important building block in further attempts to increase the pollutant removal efficiency of constructed wetland water treatment systems.

The sediment core profiles will also be used to determine the amounts and chemical forms of Se building up in the sediments over time. The potential bioavailability and eco-toxicity of residual Se forms accumulating within the CWTS ecosystem will be assessed by speciating Se in sediments, plants and other biota using high-energy synchrotron-based x-ray absorption spectroscopy (XAS). This research will be conducted using the sediment cores and biota tissue samples over time for the measurement of total Se and determination of the different chemical forms of Se. The XAS measurements will be conducted at the Stanford Synchrotron using beamtime access already approved by SSRL. The XAS research will be supervised by Dr. Soo In Yang who has considerable experience and expertise in carrying out this type of research.

3. Field Sampling and Protocols

Sampling and analysis - team responsibilities and logistics: The UC Berkeley CWTS research team will consist of two post-doctoral researchers who will make monthly sampling visits from UC Berkeley to the Salton Sea to monitor changes in the algal and cattail treatment cells. A sample coding system will be used to ensure that the field samples could be identified and tracked accurately through all the processing stages (see *Attachment 8, Table 2*). The on-site visits will take 3 days after which time the researchers will return to the UC Berkeley laboratory with the collected samples to complete chemical and other analyses, e.g., determination of Se volatilization rates of collected water samples from the algal cells. Standardized laboratory techniques will be followed to ensure both the reproducibility and accuracy of the chemical analyses. Speciation of residual Se in sediment, plant and other biota will be conducted at the Stanford Synchrotron.

Influent and effluent water sampling: On a monthly basis, influent and effluent water sampling for total Se, N, P and S will be carried out for each of the wetland cells. The sampling will consist of 250 ml grab samples according to the Bottle Submersion Method (Brynes, 1994). The acid-rinsed 250-ml polyethylene sample bottle will be lowered by hand or extension rod into the water. The sample will be preserved immediately after sampling by acidifying with Trace Metal Grade concentrated nitric acid (HNO_3) to pH < 2. After acidification, the sample will be transported at ~ 4˚C for immediate laboratory analyses. The concentrations of the elements will be determined on an unfiltered sample after vigorous digestion.

Pore water sampling: The sampling for pore water Se in each cattail cell will be conducted on a 2 m x 20 m grid system. Each unacidified Se sample will be filtered by passing through a 0.1-µm membrane filter using a 10 cm-long Rhizon Soil Moisture Filter, which is inserted into the sediment and attached to a 20-ml vacuum tube and allowed to fill. This sample will provide a sterile unacidified sample of pore water from the composite 0-10 cm sediment profile. The sample will be preserved immediately after sampling by acidifying with Trace Metal Grade HNO_3 to pH < 2. Once acidified, the samples will be transported at ~ 4˚C for laboratory analysis.

Wetland cattail: The biomasses of cattail shoots and roots will be collected every three months from three randomly selected 1m x 1m quadrats. Whole cattail plants will be removed from the wetland area with the use of a spade and representative shoot samples (i.e., actively-growing green leaf tissue) removed. Complete shoots will also be collected for seasonal biomass measurements. The below-ground portion (i.e., root sample) of the whole cattail will be extracted from the soil and rinsed on-site to remove sediments. Grab samples of standing and fallen cattail litter, seed and seed heads will be collected during the fall and winter months, which will also include dead leaf litter (standing and fallen). During the growing season, cattail samples will consist of green shoot (leaf and stem) and root tissues. Collected cattail samples will be packed and preserved ~ 4˚C for immediate transport to the laboratory. Wetland cattail biomass estimates per sampling area (1m x 1m) will be determined with randomly selected whole shoots of cattails (Kufel, 1991). Per unit area frequency of cattails will be calculated from replicated counts. The individual cattail biomass measurements will provide an estimate of the total above-ground seasonal biomass per unit area.

Algae sampling: During the monthly visits, samples from the algal cells will be collected using the Bottle Submersion Method. The appropriate number and quantity of samples will be determined by the algal biomass produced under field conditions. At least replicate 1 L samples will be collected from each algal treatment cell. The collected algal samples (i.e. water containing the algae) will be frozen and transported for laboratory analysis. Algal growth and population density will be assessed monthly by measuring biomass dry weight and comparing it with extracted chlorophyll contents. In the laboratory the algal biomass samples will be processed with acetone and DMSO to extract and measure total chlorophyll by spectrophotometric methods. Other analytical methods will also be performed on water and algal biomass samples.

Biological specimens: Biological samples, i.e., biofilms, macroinvertebrates, fish and amphibians, from the CWTS will be collected every three months and preserved on ice and transported to our laboratory for total Se analysis and Se speciation by XAS. Macroinvertebrates will be surveyed seasonally using Hester-Dendy multilevel benthic colonization plates (Hester and Dendy, 1962) and emergence trap collection (Downing and Rigler, 1984). Multiple collection sites will be located in different habitats near the inflow, middle, and outflow of the Alamo River wetlands. The Hester-Dendy colonization plates will be deployed on the substrate and placed in plastic bags upon retrieval. Invertebrates collected with the emergence traps will be trapped in a plastic jar containing 100 ml of 50% ethyl alcohol. Collected invertebrates will be identified in the laboratory. Specimens were identified to family or genus level using Merritt and Cummins (1996). Fish and amphibians will be sampled in the spring and fall using nine minnow traps in the wetland as described in Cochran (1998). The minnow traps will be set up for 28 days and checked three times each week on the same days to standardize the study. Each captured fish and amphibian will be placed in the bucket and identified on the site or in the laboratory.

Sediment sampling: Sediment sampling will be based on a 2 m x 20 m grid system in each cattail treatment cell. This sampling will be performed with a Cole-Palmer sediment core sampler (10 inch long x 1 inch diameter) with a replaceable butyrate liner. This will provide an *in situ* measurement of sampled sediment with near-zero profile contamination. The wetland area to be sampled will be first cleared of plant matter. The thin-walled probe will be inserted into the sediment to just beyond the desired depth (15 cm). The probe will then be removed from the sediment, and the liner will be removed from the probe according to Boulding (1994). The liner containing the sediment profile sample will be capped and stored on ice for transport to the laboratory.

Physicochemical parameters: At each monitoring visit, the physicochemical surface water parameters that may affect the availability and uptake of Se and other nutrients by cattail and algae in the CWTS will be measured *in situ* in the inflow and outflow of each treatment cell. We will measure surface water pH, electrical conductivity, temperature, dissolved oxygen, and salinity. Three replicates will be measured for each parameter using a Hanna Instruments HI 9828/4 Multiparameter Water Quality Portable Meter (Woonsocket, RI, USA) to properly assess the effect of mechanical alternations in the environmental conditions.

Selenium mass balance: Sources and sinks of Se in the algal cell and cattail cell, and a Se mass balance, are summarized in *Attachment 8, Figures 4 A and B.* The total Se input and output from each cell will be estimated from total Se measurements in the inflow and outflow. The mass of Se stored in the water and sediment components (i.e., mineral soil and litter) will be estimated as the product of their respective volumes and concentrations. Selenium biomass accumulation will be estimated using the mean Se concentrations measured in cattail shoot and root tissues and algal biomass. The rates of Se volatilization will be calculated as the difference between the net input of Se and the mass left in the system.

Water budget: The water budget will be calculated as the difference between total water input and output. The input will include inflow (pumped river water), rainfall [and ground water] while the total water output includes outflow, seepage and evapotranspiration (see *Attachment 8, Figure 5*). The following general equation is used to determine a water budget for each CWTS cell:

$$I + P + G - O - ET - S = 0$$

where,

I = inflow (pumped river water)
P = precipitation
G = ground water recharge
O = outflow
ET = evapotranspiration
S = seepage

Inflow and outflow measurements in each cell will be made based on two important variables: 1) water level of the treatment cell, and 2) the crest elevation of the weir installed at the outlet of each cell. These data are then used with the Kindsvater-Shen equation that relates water head to rate of outflow as follow:

$$Q = 0.121\ C \tan\left(\frac{\theta}{2}\right)(h + k)^{5/2}$$

where,

> Q = Discharge (m^3/s)
> C = Discharge coefficient
> θ = Notch angle
> h = Head (m)
> k = Head correction factor (0.001 m)

Local precipitation and evapotranspiration data will be obtained from the Calipatria CIMIS station of the California Irrigation Management Information System (CIMIS). In addition, seepage will be estimated by the budget equation mentioned above.

4. Analytical Methods and Procedures to be used in the Laboratory

Quality assurance and control: Analytical methods and protocols will be conducted using standard quality procedures, i.e., certification of operator competence, recovery of known additions, analysis of externally supplied standards, duplicates and reagent blanks, and calibration with standards.

Water analysis methods: Dissolved Se and S will be measured directly from water samples with inductively coupled plasma-dynamic reaction cell-mass spectrometry (ICP-DRC-MS) and atomic absorption spectrometry (AAS) according to EPA and Standard Methods (EPA 3010A, 1992) after acid-digestion. For total N and P will be analyzed using QuikChem IV automated system and Lachat methods according to EPA instruction (1983).

Sediment analysis methods: Sediments will be removed from capped butyrate liners, cut into 5 cm profile sections (0-5, 5-10 and 10-15 cm), air-dried, ground, and acid-digested with HNO_3/HCL followed by measurement for all trace elements of interest with ICP-DRC-MS.
Total Se analysis method in biological samples: Wetland biological tissue samples will be dried at 70˚C, weighed, ground in a Wiley Mill to pass a 40-mesh screen, and samples will be acid-digested with HNO_3/HCl (Zarcinas et al., 1987). Total Se will be measured with ICP-DRC-MS.

Evaluation of Se ecotoxicity (Se speciation): Collected sediment, plant, algae, biofilm, benthic invertebrates, insects, fish, and bird excreta samples will be transported into the lab under the refrigerated conditions. All the samples will be ground using pestle and mortar in liquid nitrogen and then packed into 2mm X-ray absorption spectroscopy (XAS) sample holders followed by preservation at -80 °C. The prepared samples will be transported on dry-ice to the beamline 7-3 of the Stanford Synchrotron Radiation Lightsource. Se speciation study will be conducted using X-ray absorption near-edge structure and extended X-ray absorption fine structure at Se K-edge range. All the samples will be measured inside of helium-filled cryostat maintaining the temperature at approximately 10 Kelvin (-263.15 °C) to minimize atomic thermal vibrational effect in defining Debye-Waller factor and radiational damage to the samples. The collected raw data will be processed using a suite of XAS program, EXAFSPAK (George and Pickering, 2001). A typical XAS beamline setup is shown in *Attachment 8, Figure 6*.

Statistical analysis: All data will be analyzed using PASW Statistics 18 to conduct factorial ANOVA and Multiple regression analyses. Factorial ANOVA tests will be used to determine the influence of plant species, organic material, wet-dry cycling and retention time (sampling period) and their interactions on Se removal, Se volatilization and Se distribution. All tests will be conducted at a 95% confidence interval ($\alpha = 0.05$). Significant differences will be reported at $p \leq 0.05$, and sample variance will be expressed as the standard error.